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Continuous *In Vivo* Monitoring



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2

Electrochemical Sensors for in vivo Glucose Sensing

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2.1 INTRODUCTION

The need for implantable glucose sensors allowing continuous monitoring of physiological glucose levels has been recognised for more than a quarter of a century. It is generally agreed that for patients with Type I diabetes mellitus,

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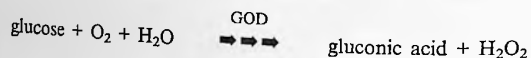
only continuous monitoring of glucose levels will permit something approaching normoglycaemia (Tchobroutsky, 1991), thus preventing both short-term and long-term complications (for example, hypoglycaemic episodes in the former case and retinopathy, neuropathy and nephropathy in the latter). However, as shown in the DCCT trial (DCCT, 1991), improved normoglycaemia control is often accompanied by a greater risk of severe hypoglycaemia, due to the so-called hypoglycaemia unawareness syndrome. Continuous glucose monitoring must also seek to decrease this risk.

The control of glucose levels by an artificial device was first demonstrated by Albisser *et al.* (1974): similar performance with specifically an implantable sensor was shown by Shichiri *et al.* (1982, 1983, 1984). These landmarks have been followed by an impressive number of papers developing various concepts for implantable and extracorporeal glucose sensors. The ultimate goal is the development of a closed-loop insulin delivery system, where insulin would be delivered by a pump controlled by a glucose sensor. However, there are many other situations where an implantable glucose sensor could be very useful for monitoring insulin-dependent diabetics or any patient with unstable body chemistry. Examples would be a overnight alarm for hypoglycaemia, or a monitor during surgery, intensive care, childbirth and initiation and modulation of insulin therapy. All these applications are of a limited duration and, considering the present state-of-the-art of the implantable glucose sensors, constitute the most realistic targets for the first stage of continuous, intracorporeal glucose monitoring. Recent consensus shows a short-term hypoglycaemia alarm to be the first clinical priority.

Many different strategies for potentially-implantable glucose sensors have been proposed. However only a few have so far been submitted to serious *in vivo* evaluation. These are principally the electrochemical devices, and more specifically amperometric enzyme electrodes using glucose oxidase (GOD) immobilised on an amperometric transducer. Enzyme electrodes coupled with microdialysis have been also investigated. Microdialysis is an *in vivo* sampling technique allowing a continuous sampling of metabolites from blood or interstitial fluid by infusing a tissue via a semipermeable, double channel catheter. By coupling the dialysate from a microdialysis probe to a glucose sensor, extracorporeal glucose monitoring can be performed. The principles of glucose sensors are described briefly below. The emphasis is on those sensor aspects that are directly linked to *in vivo* applications.

2.2 ENZYME ELECTRODES FOR GLUCOSE

Glucose oxidase catalyses the following reaction



and this can be monitored using either potentiometric or amperometric techniques. Despite considerable research effort on potentiometric systems based on glucose ENFETs (enzyme field effect transistors, see Section 7.6.2), no *in vivo* feasibility has been demonstrated, due to the difficult problem of the variation in the buffer capacity of the enzymatic membrane, as well as that of the biological fluid.

The amperometric approach dominates glucose sensing and was first proposed by Updike & Hicks (1967). Using a two- or three-electrode electrochemical cell, either oxygen consumption or peroxide formation can be monitored. In both cases, an external potential is applied and the resulting current is measured. This, for a well designed sensor, is directly proportional to the glucose concentration. An obvious problem with this scheme is the requirement of oxygen as a co-substrate. To circumvent this, several so-called "mediators" have been investigated: these act as a substitute for oxygen. Ferrocene and its derivatives are the most commonly used (Claremont *et al.*, 1986). These diffusional mediators decrease the influence of the local oxygen concentration. Moreover, by allowing lower working potentials (100–200 mV relative to the Ag/AgCl electrode compared to 600–700 mV for direct peroxide detection), the problem of electroactive interferences is also minimised. However, leaching of diffusible mediator results in a loss of sensitivity and more importantly, when used *in vivo*, the high risk of introducing the mediator into the body. These problems have led to the abandonment of this approach for *in vivo* devices. Recently, "electrical wiring" of the enzyme has been employed, by which the enzyme is associated with the electrode via a long polymer having a dense array of covalently-attached mediator groups (Csöregi *et al.*, 1994).

2.3 CRITERIA FOR BIOSENSOR PERFORMANCE

Besides the obvious requirement concerning safety to the patient, which is of primary concern for an *in vivo* use, other functional requirements of implantable sensors can be divided into general requirements common to all sensors and specific requirements for *in vivo* use. General parameters include specificity, linearity in the required concentration range, adequate response time, detection limit and resolution, acceptable signal/noise ratio, reasonable storage and operational lifetime. Specific factors include miniaturisation, sterility, independence of hydrodynamic and oxygen variations, accuracy in the normal glucose concentration levels and stringent demands on biocompatibility. A number of these issues are addressed below.

2.3.1 THE TRANSDUCER

To decrease the overall sensor size, a two electrode set-up, with a Pt, Pt-Ir or Pt-black working electrode, has been most commonly used. The second

electrode, which in this case serves as both the reference and counter electrode, is usually an Ag/AgCl wire but can even be an external electrode, i.e. attached to the skin surface. The fabrication process uses thin metallic wires, i.e. each sensor is handmade.

Notably, two sensors described in the literature are based on microfabricated, thin-film, three-electrode transducers on either a polyimide (Mastrotaro *et al.*, 1991) or a silicon (Gernet *et al.*, 1989) substrate. The former consists of Pt-black working and counter electrodes while the latter uses simple Pt thin films. In both cases, a thin film Ag/AgCl reference electrode is used. Another approach has been based on microcontainment technology, where the Pt thin film electrode is in a cavity (500 μm diameter), which is then filled with an enzyme membrane (Steinkuhl *et al.*, 1994): this sensor was later used in a microdialysis system (Steinkuhl *et al.*, 1996). Another transducer design was used in the sensor based on oxygen detection reported in (Armour *et al.*, 1990). This was composed of two Pt wire electrodes and an Ag/AgCl reference. These were integrated into a flexible, silicone rubber catheter. Silicone, being highly permeable to oxygen but impermeable to glucose, allows the relative ratios of glucose to oxygen diffusion to be modulated by adjusting the respective areas of the enzyme layer/silicone. This sensor was used in a differential mode against a reference O_2 sensor, which was similar to the glucose sensor but lacked the enzymatic membrane.

2.3.2 THE ENZYME MEMBRANE

In the vast majority of sensors, the GOD (usually in large excess), either alone (Kerner *et al.*, 1993; Pickup *et al.*, 1993) or mixed with bovine serum albumin (BSA) (Gough *et al.*, 1985; Bindra *et al.*, 1991; Moussy *et al.*, 1994; Gernet *et al.*, 1989), is immobilised by glutaraldehyde cross-linking. By adjusting concentrations of various components, gel-like membranes having good mechanical stability and thickness of several μm are formed. In some cases, catalase is co-immobilised with GOD in order to decrease inactivation by H_2O_2 (Tse & Gough, 1987). GOD immobilisation on Sepharose has also been described (von Woedtke *et al.*, 1994a,b).

Less conventional enzyme immobilisation can be performed by galvanostatic electrodeposition of a layer of GOD and BSA of about 5 μm thickness, which is later crosslinked with glutaraldehyde (Johnson, 1991). This method allows spatial control of the enzyme deposition, which is an important advantage for miniature sensor fabrication.

For the "enzyme-wired" electrodes, the conducting redox hydrogel was formed by poly((vinylimidazole)Os(bipyridine) $_2\text{Cl}$) $^{+2+}$ redox polymer, complexed with GOD and crosslinked with poly(ethyleneglycol) diglycidyl ether (Csöregi *et al.*, 1994).

2.3.3 THE INFLUENCE OF OXYGEN

The problems which may arise from low and non-uniform oxygen tensions in the subcutaneous tissue have motivated the development of sensors using mediators (Claremont *et al.*, 1986) or sensors with vascular implantation sites (Armour *et al.*, 1990). In the subcutaneous tissue, oxygen pressures vary in the range 20 to 30 mmHg (Fischer *et al.*, 1989; Zhang & Wilson, 1993) corresponding roughly to 1×10^{-5} M, whilst the glucose concentration can rise to 2×10^{-3} M. Therefore the risks of a non-linear glucose response and/or response saturation are very high. The most common approach for avoiding or at least decreasing the oxygen influence on the sensor signal is the use of an outer, diffusion-limiting membrane with properties that are designed to reduce diffusion of glucose with respect to oxygen (Shichiri *et al.*, 1983, 1984). Beside lowering the oxygen dependence, this membrane has several other effects on the sensor behaviour such as the minimisation of influence of enzyme- and electrode kinetics on the sensor response, a lower temperature coefficient and greater linear range (increase of the apparent K_m in the diffusion-limited regime). It serves also as protection for the sensor, and since it is the outer membrane, its properties with respect to biocompatibility and stability in biological fluids will largely determine the functional characteristics of the sensor. Polyurethane (PU) membranes are the most frequently used, followed by Nafion (Moussy *et al.*, 1994), perforated polyethylene (von Woedtke *et al.*, 1994a,b) and photopolymerisable derivatised poly(ethylene oxide) (Csöregi *et al.*, 1994).

2.3.4 BIOCOMPATIBILITY

This has been defined as the ability of a material to perform with an appropriate host response in a specific application (Braybrook, 1993). It is a dynamic and two-way process that involves the time-dependent effect of the biosensor on the host and *vice versa*. Understanding the pathophysiological mechanisms is becoming critical at the present stage of the sensor development, and these are treated in more detail in section 1.4.3 and Chapter 5. Biocompatibility will ultimately determine the feasibility of clinical use, not only in terms of the presence or absence of adverse reactions (toxicity, acute and chronic inflammatory and immune reactions) but also because the host-sensor reaction will determine sensor function (drift, response time and operational lifetime).

One of the rare studies of the toxicity of biosensors has been performed on glutaraldehyde-crosslinked membranes (Zhang *et al.*, 1991). *In vitro* cell culture tests have shown that freshly-prepared, ethylene oxide sterilised sensors showed moderate toxicity, mainly due to the presence of non-reacted glutaraldehyde. If a polyurethane polymer membrane was present, residual solvents also leached. However, the toxic effect was removed by soaking the

sensor for about one week in phosphate buffer. Immunogenicity of sensors implanted in rats has been tested by detecting specific IgG-antibodies produced against various components of the sensor such as cellulose acetate, polyethylene, polyurethane and enzyme membrane (Ziegler *et al.*, 1994). The highest immune response was observed with cellulose acetate and regenerated cellulose, which, however, are usually used as inner, anti-interference layers and thus are not in direct contact with the tissue. The polyurethane was shown to be of low immunogenicity, and no cytotoxicity was observed *in vitro* using monolayer cell cultures. The biocompatibility studies of subcutaneously-implanted, alcohol-sterilised Nafion membranes have shown the rapid onset of fibrosis, resulting in the formation of a fibrous capsule (Turner *et al.*, 1991). However, the thickness of the capsule remained relatively thin ($<100\text{ }\mu\text{m}$ after more than three months) and well vascularised with a near steady-state achieved after 43 days. A different situation was observed for intravenous implants where fibrosis progressed steadily up to at least 100 days. However, no chronic inflammation was observed. It was concluded that the biocompatibility of Nafion was comparable to that of silicone rubber.

2.3.5 THE SITE OF SENSOR IMPLANTATION

This is a critical consideration. Implantation in the vascular space would be the most straightforward choice, since most clinical information deals with blood glucose levels. So far, only one sensor has been successfully implanted in the vascular space (Armour *et al.*, 1990). The major concern over thrombosis and infection has forced another choice of implantation site, namely the subcutaneous tissue. This site has been validated using reference methods such as microdialysis (Jansson *et al.*, 1988; Bolinder *et al.*, 1989) or a wick technique (Fischer *et al.*, 1987; Brückel *et al.*, 1989) by showing that the slow changes in blood glucose concentrations were reflected with an acceptable time lag (5–10 min) in the subcutaneous tissue. A more complicated situation (longer time lag or a complete lack of blood/tissue equilibration) can exist for faster variations of glycaemia. Despite this doubt, and the controversy surrounding the precise glucose concentration in extracellular fluid, the subcutaneous tissue is presently the favoured site of implantation. Other reasons to favour the subcutaneous site are that it is already used for implantation of needles for insulin perfusion, and that percutaneous access is more convenient if a periodic exchange of sensors is envisaged.

2.3.6 CALIBRATION AND IN VIVO SENSITIVITY

The initial results obtained with the subcutaneously implanted sensors were expressed in nA without any transformation of the sensor current output into a glucose concentration. Attempts to use the *in vitro* sensitivity to calculate the subcutaneous glucose concentration gave confusing estimates, which varied

between 20 and 95% of the blood glucose levels. This showed clearly the necessity of *in vivo* calibration for subcutaneous monitoring.

Using two steady-state conditions for low and high blood glucose levels (basal/insulin-induced hypoglycaemia, and glucose infusion/bolus hyperglycaemia respectively), a two-point *in vivo* calibration method has been used (Poitout *et al.*, 1993). The *in vivo* sensitivity and the background current (i.e. the current that would be obtained in the absence of glucose) were determined from (respectively) the slope and the intercept of a straight line representing current vs. blood glycaemia. The *in vivo* sensitivity was then used to calculate an apparent subcutaneous glucose concentration and an excellent correlation between blood and subcutaneous glucose levels was found. These results were confirmed in the same animal model (the rat) using the same technique with constant hypo- and hyperglycaemic plateaux (Koudelka *et al.*, 1991). The two-point calibration method was also used in non-diabetic patients, where the basal and plasma glucose levels after an oral glucose load were paired with the concomitantly measured sensor currents (Pickup *et al.*, 1993). It was also used for diabetic patients, where the glucose levels were adjusted by feedback-controlled dextrose and insulin infusions (Kerner *et al.*, 1993). The possibility of using a one-point calibration was investigated (Bobbioni-Harsch *et al.*, 1993; Csöregi *et al.*, 1994): this is only valid if the background current is assumed or observed to be small compared to the sensitivity. More complicated calibration methods based on both *in vivo* and *in vitro* sensor parameters have been used (Johnson *et al.*, 1992; Gilligan *et al.*, 1994). The *in vitro* calibration adjusted for oxygen sensitivity (against a reference oxygen sensor) was employed in the case of oxygen-based sensors implanted in the dog vena cava (Armour *et al.*, 1990).

In most cases, *in vivo* studies using different animal models or humans show that the *in vivo* sensitivities were almost always lower compared to the *in vitro* ones, and that the background currents were higher. An exception is the intravascularly implanted sensor where the original pre-implantation sensitivity was used throughout the test (Armour *et al.*, 1990). Pre-implantation sensitivity for subcutaneously implanted sensors has been used in the initial stages of one experiment (Updike *et al.*, 1994). The reasons for this impaired *in vivo* sensitivity are not definitely known, although several explanations have been proposed. The most likely is the formation of a protein or cellular coating at and around the sensor, hindering glucose diffusion. The frequently observed re-establishment of the initial, *in vitro* sensitivity following the sensor explantation and rinsing may confirm this hypothesis (Rebrin *et al.*, 1992; Gilligan *et al.*, 1994; Thomé-Duret *et al.*, 1996). Scanning electron micrographs of explanted sensors showed that while functioning sensors had minimal visible coating of cellular or other material, the non-functioning ones had thick deposits and damaged sensor membranes (Pickup *et al.*, 1993). However, in this paper, no information was given on whether the pre-implantation sensitivity was re-established *in vitro* after explantation. The

extent and the time evolution of this unavoidable sensor-host interaction, resulting in the outer membrane fouling and/or a fibrous capsule formation, will depend strongly on the biocompatibility of the sensor.

Another hypothesis suggests the inhibition by as yet unidentified low molecular weight substances present in subcutaneous extracellular fluid and plasma, which diffuse through or adsorb onto the polyurethane membrane (Kerner *et al.*, 1993). The so-called inhibition factor "Q" leads to a drift in sensor signal and finally to sensor deactivation. Also in this case, the sensitivity of the explanted sensors was rapidly restored in a buffer solution. The participation of the peroxide in the biodegradation of polymer materials, resulting in stress-cracking and leaching of small molecular weight substances was discussed by Updike *et al.* (1994). During an inflammatory reaction, the exudate has been shown to have lower glucose concentrations (Rebrin *et al.*, 1992) than the interstitial fluid: this might also contribute to the impaired *in vivo* sensitivity. However, in most of the previously cited work, no visible inflammatory reaction or bleeding was observed. The different components of biocompatibility—physicochemical interactions at the interface, material degradation, and local and systemic host response will ultimately determine the longevity and performance of implanted sensors. This stresses the importance of these issues in sensor development (Kyröläinen *et al.*, 1995). A successful transition from animal models to clinical investigation implies the solution of these problems.

2.3.7 INTERFERENCES

The problem of electrochemical interference is particularly acute at the high anodic potentials required for hydrogen peroxide oxidation. To date, the effects of the few most likely interferents have been studied: endogenous species such as ascorbate and urate, and exogenous species such as paracetamol (acetaminophen). The outer membranes used on sensors provide minimal protection only. Inner, low molecular weight cut-off (<200 MW) membranes of cellulose acetate (Brückel *et al.*, 1989; Bindra *et al.*, 1991) or electropolymerised poly(o-phenylenediamine) (Moussy *et al.*, 1994) have been investigated. Both are effective in screening out the endogenous interferences at the physiological levels. The paracetamol problem is more complicated (Moatti-Sirat *et al.*, 1992b). To date, the most efficient paracetamol screening was obtained using a membrane composed of several alternating layers of cellulose acetate and Nafion (Moatti-Sirat *et al.*, 1994; Zhang *et al.*, 1994). Elimination of interferences by peroxidase-catalysed oxidation was performed by Csöregi *et al.* (1994). The limited life-time of this layer led to a different approach in which electro-oxidation of the interferents was avoided by using a low (95 mV vs. SCE) operating potential (Csöregi *et al.*, 1995).

2.3.8 STERILISATION

Although most of the initial animal experiments were performed with non-sterile devices, device sterility is obviously a prerequisite for all human intracorporal measurements. More recently, chemical or irradiation sterilisation procedures have been employed. For the former, glutaraldehyde (Kerner *et al.*, 1993; Pickup *et al.*, 1993) or ethylene oxide (Poitout *et al.*, 1993) were used. The question of the remaining toxic residues after chemical sterilisation needs to be clarified. The irradiation sterilisations consisted either of a 2.5 MRad e-beam (Johnson *et al.*, 1992) or gamma dose (Koudelka *et al.*, 1991; Bobbioni-Harsch *et al.*, 1993). A lower, 0.65 Mrad dose of gamma-irradiation combined with several days' exposure to a peroxide solution has been proposed (von Woedtke *et al.*, 1994b). Complete telemetric and sensor assembly sterilisation has also been performed using a dilute thimerosal saline solution for 24 hours (Gilligan *et al.*, 1994).

2.4 *IN VIVO* EVALUATION OF IMPLANTABLE SENSORS

Ten years ago (Shichiri *et al.*, 1986), the successful use of a subcutaneously-implanted glucose sensor coupled to an insulin or glucagon infusion pump was described. By exchanging the sensor after four days, normalised glycaemic profiles of diabetic subjects were obtained for one whole week. One is tempted to ask how far the state of the art has progressed since then. With three notable exceptions (Armour *et al.*, 1990; Gilligan *et al.*, 1994; Updike *et al.*, 1994), all other *in vivo* sensor evaluations were performed for periods ranging only from a few hours to several days. At this point, one might pose a fundamental question about the feasibility of *in vivo* glucose monitoring. The answer is far from being straightforward and a differentiation between short-term (up to several days, a week) and long-term (several months, a year) applications should be made. The use of a sensor *per se* (e.g. in a hypoglycaemia alarm without controlling insulin delivery) is of real clinical and economical importance. Thus, even though it may be considered as a step back in the scheme of *in vivo* glucose monitoring, the development of a night-time hypoglycaemic alarm is entirely justified (Pickup, 1993). In addition, as already mentioned in the introduction, other short-term uses of implantable glucose sensors would be also of clinical interest.

In all studies but one (Armour *et al.*, 1990), sensors were implanted subcutaneously. Most of the tests describe short-term glucose monitoring, and were conducted on animals such as rats (Moatti-Sirat *et al.*, 1992a; Bobbioni-Harsch *et al.*, 1993; Csöregi *et al.*, 1994), dogs (Poitout *et al.*, 1992; Rebrin *et al.*, 1992; Moussy *et al.*, 1994), rabbits (Johnson *et al.*, 1992) and sheep (Brückel *et al.*, 1989). It is impossible to compare the results obtained because

not only were different sensor designs and different animal models used but also different protocols were applied: glucose infusions, intraperitoneal glucose loads and glucagon-insulin administration, different blood withdrawal sites for the reference measurements and different implantation sites, etc.

One of the rare observations common to most of these experiments was the so-called run-in period, i.e. the time after implantation necessary to obtain a stable current output. However, the duration of this period, during which the current decreases and then stabilises, varied from one hour (Bobbioni-Harsch *et al.*, 1993; Moussy *et al.*, 1994), to 2–4 hours (Johnson *et al.*, 1992; Kerner *et al.*, 1993) to overnight (Moatti-Sirat *et al.*, 1992a). A second common observation of all these short-term animal experiments was the post-implantation decrease of the sensor sensitivity, although the extent varied. In some cases, the *in vivo* sensitivity first decreased but then remained relatively stable for implantations of several days (Moatti-Sirat *et al.*, 1992a; Bobbioni-Harsch *et al.*, 1993). Following an *in vivo* calibration, the *in vivo* sensitivity was used to calculate *a posteriori* the subcutaneous glucose levels, which were compared with the reference blood or plasma glucose concentrations. The graphical representation of the correlation plots was performed either by simple regression lines or by the more clinically oriented Clarke error grid analysis (Clarke *et al.*, 1987). In the former, the accuracy of data is expressed by a correlation coefficient, while in the latter, a percentage difference related to the clinical requirements for accuracy is used. Thus, more clinically meaningful evaluation of the accuracy between the sensor values and the reference glucose values is obtained (Moatti-Sirat *et al.*, 1992a).

The results of the short-term animal studies confirmed previous results (for the pre-1988 situation, see Pfeiffer & Kerner, 1988), demonstrating that a subcutaneously implanted sensor can follow correctly the variations of glycaemia for several days. Automated feedback control of the subcutaneous glucose concentration in diabetic dogs before and during an OGTT has been demonstrated (Rebrin *et al.*, 1989). In this paper, the relatively high failure rate of the subcutaneously implanted sensors was correlated with the problem of tissue reaction. The results of the animal experiments have also been confirmed in non-diabetic patients (Johnson *et al.*, 1992; Pickup *et al.*, 1993; Poitout *et al.*, 1993). Tests lasting up to 72 hours showed good correlations between the subcutaneous glucose concentrations and blood glucose values during standard glucose tolerance tests (OGTT) or normal meals (Johnson *et al.*, 1992).

As already mentioned, *in vivo* calibration was performed *a posteriori*, i.e. upon completion of the experiment. Although acceptable for initial sensor evaluations, this is completely unacceptable for clinical applications. A wearable control and monitoring unit with built-in software allowing real-time *in vivo* calibration has been described (Poitout *et al.*, 1993). A two point calibration was used to determine the *in vivo* sensitivity—at basal level, and at

a value 60 min after an OGTT (which corresponds to a slightly increased glucose plateau in non-diabetic patients). Performed 14 hours after implantation, the sensors could reliably follow the plasma glucose levels following the OGTT.

The longer-term evaluations have been performed in dogs, using either a catheter-shaped, intravascularly-implanted sensor (Armour *et al.*, 1990) or a modified Direct 30/30™ sensor implanted in the subcutaneous tissue (Gilligan *et al.*, 1994; Updike *et al.*, 1994). The former uses an enzyme electrode of two-dimensional construction (flexible silicone rubber catheter with an enzyme membrane at the tip) based on oxygen detection, while the latter is based on peroxide detection, using a three-electrode transducer and commercially available, polyurethane-based anti-interference and enzyme membranes. Both use implanted telemetry systems, either near the electrode (Armour *et al.*, 1990) or integrated with the electrode (Shults *et al.*, 1994). The telemetry systems are not small; dimensions of $5 \times 5.5 \times 1.1$ cm for the former and $7 \times 3.2 \times 1.2$ cm for the latter, with weights of 27 g and 70 g respectively.

The catheter-type oxygen-based glucose and a reference oxygen sensor used in the differential measurement mode was evaluated during several weeks' implantation in the *vena cava* of non-diabetic dogs (Armour *et al.*, 1990). In total, six sensors were implanted so that they free-floated in the blood stream: five had telemetry and one had percutaneous leads. After a healing period of 1–2 weeks, the sensor performance was assessed using various glucose infusion protocols. Results obtained on different days after implantation show that sensors could indeed track the glucose variations using their pre-implantation calibration. Different failures related to electronics and wire-breaking complicated the assessment of sensor lifetime. The longest implantation was 108 days, and this particular sensor was still functional upon explantation, with minimal changes in post- vs. pre-implantation *in vitro* sensitivity. Except for one dog, in which the sensor adhered to the vessel and was encapsulated with a thin layer of tissue, no adherent clots or necrotic regions were found in post-implantation examinations.

The second longer-term evaluation concerns the sensors integrated within a telemetry system which were implanted in the subcutaneous tissue of dogs. The run-in period (instability of the sensor signal upon implantation) up to 18 days was associated with inflammation followed by the development and maturation (vascularisation) of the foreign body capsule. A stable sensor output was then obtained, and glucose monitoring in freely-moving dogs was performed with three sensors from 20 to 94 days using intravenous glucose infusions (Updike *et al.*, 1994). It was observed that the sensor lifetime was limited by the biodegradation of the polyurethane membrane. The addition of a bioprotective membrane (material not disclosed) seemed to improve the sensor lifetime (Gilligan *et al.*, 1994). In this case, 10 implanted sensors were evaluated, for durations ranging from 20 to 114

days. An important observation concerned the decrease of vascularisation of the fibrous capsule after about 3 months, resulting in oxygen limitations (>15 mM glucose). This confirms the importance of stable vascularisation. The feasibility of long-term glucose monitoring in the subcutaneous tissue would thus depend on the formation of a defined and well-vascularised tissue capsule, characterised by stable glucose and oxygen access to the implanted sensor. What the duration of this unstable period is, and whether it is acceptable to patients, has yet to be determined. This will depend on the sensor's subsequent functional lifetime. For example, if the sensor functions for at least several months or one year, a run-in period of a few days or weeks respectively might well be acceptable.

2.5 OTHER IMPLANTABLE ELECTROCHEMICAL SENSORS

The sensor developed by Wilkins *et al.* (Atanasov & Wilkins, 1993) differs from the classical enzyme electrodes described so far, although it is also an enzyme electrode. It consists of two parts—an amperometric peroxide detector of a wire-type and an enzyme microbioreactor—enclosed in a 1 cm-diameter plastic tube. The microbioreactor is filled with fine carbon powder, onto which GOD has been immobilised. Nafion-pre-treated polycarbonate is used as a diffusion limiting membrane. The sensor housing provides an inlet and an outlet, so that the reactor can be refilled when necessary with a fresh carbon/enzyme suspension. This approach addresses the problem of loss of enzyme activity over time. The device is connected to a miniaturised potentiostat and signal transmitter, and has been tested in plasma and whole blood *in vitro*. Very preliminary, short-term, subcutaneous implantation in a sheep has also been performed.

A completely different type of sensor is the electrocatalytic device. This is based on direct, anodic oxidation of glucose at Pt-based electrodes. The electrochemical cell is usually completed by a Pt counter and an Ag/AgCl reference electrode. Due to the complexity of the resulting electrochemical signal, complex measurement methods have to be used to extract a meaningful glucose signal: charge-compensated cyclic voltammetry (Sarangapani *et al.*, 1988), impedance (Preidel *et al.*, 1990) or differential pulse voltammetry (Lemke & Lustermann, 1991). The measurements are complicated by the adsorption of the reaction products and various other substances, causing a rapid fouling of the electrode surface, with concomitant loss of sensitivity. Thus, despite the option of using a protective membrane, frequent electrochemical reactivations of the electrode have to be performed. The short-term, *in vivo* evaluations of the electrocatalytic sensors were performed in dogs using catheter-type sensors implanted either intravascularly or subcutaneously (Lemke & Lustermann, 1991). An electrocatalytic sensor within a flow-through cell for extracorporeal glucose monitoring was evaluated in the blood

circulation of a sheep (Preidel *et al.*, 1990). In later work, an improved design of the flow-through cell was used for longer-term experiments (Preidel *et al.*, 1993). Using intravenous glucose tolerance tests (IVGTTs), the sensor behaviour was evaluated over several weeks, with good stability of the calibration up to 33 days.

Recently, a new strategy to overcome the loss of *in vivo* sensitivity of subcutaneously implanted sensors was proposed (Rigby *et al.*, 1995). It uses slow flow (60 μ l/h) of an isotonic solution over the tip of a classical glucose sensor, to form a liquid film at the sensor/tissue interface. This film should help to reduce or eliminate the biofouling and thus to improve the sensor stability. Several such sensors were implanted subcutaneously in rats and evaluated up to four hours. Using the *in vitro* calibration values, good correlations with blood glucose levels were obtained.

2.6 MICRODIALYSIS

Microdialysis combined with an *ex vivo* glucose sensor provides an interesting alternative to the implantable glucose sensor. Microdialysis as an *in vivo* sampling technique was developed more than 20 years ago for measurement of neurotransmitters. Its role in the validation of the subcutaneous tissue implantation site was mentioned above (Jansson *et al.*, 1988; Bolinder *et al.*, 1989). The system consists of a hollow-fibre microdialysis probe, a microcell with a glucose sensor, and a precision pump. The hollow fibre (outer diameter 200–500 μ m), constantly perfused with a physiological solution, is implanted subcutaneously and connected to the extracorporeal pump. Due to the concentration gradient at the hollow-fibre/tissue interface, glucose and other small molecular weight substances (molecular-weight cut-off of the fibre is typically 20–50 kDa) diffuse into the fibre, and the dialysate then flows to the detector cell. The most important parameters that determine the system performance are the hollow-fibre length and perfusion rate. These determine the relative recovery, i.e. the ratio between glucose present in the tissue and that leaving in the dialysate. Similarly to the implantable glucose sensors, local electrochemical interferences can still be present. Several groups are currently working on continuous glucose monitoring using the microdialysis approach (Aalders *et al.*, 1991; Meyerhoff *et al.*, 1992; Moscone & Mascini, 1992; Pfeiffer *et al.*, 1993; Hashiguchi *et al.*, 1994; Steinkuhl *et al.*, 1996). All except the last-cited have been tested *in vivo* in animals, or non-diabetic and diabetic patients.

A microdialysis probe (16 mm length, 500 μ m o.d.) was implanted in 13 non-diabetic and seven diabetic patients and evaluated up to 21 hours post-implantation (Meyerhoff *et al.*, 1992). Perfusion rates of 7 μ l/min were used, and the test consisted of an OGTT for the healthy subjects and a meal for the diabetics. The sensor (a two-electrode, hydrogen peroxide detector) was

calibrated using a one-point *in vivo* calibration, using the *in vitro* background current correction. The relative recovery of the microdialysis varied for each individual, although after individual calibration, the changes of blood glucose concentrations were correctly monitored via the subcutaneous dialysate. Time-lags of up to 18 minutes were observed. The interindividual variability of the recovery was explained by differing tissue reactions and by the differences in the tissue resistance to glucose diffusion. Comparable microdialysis probes were investigated in eight diabetic patients under steady-state and dynamic variations of blood glucose levels (Keck & Kerner, 1993). Perfusion rates of up to 9 $\mu\text{L}/\text{min}$ gave good glucose recovery values, while higher rates resulted in much lower recoveries. Dynamic experiments showed that for increasing glucose concentrations, there was an interindividually variable time-lag of 10 to 40 minutes, while only short time lags were observed upon decreasing blood glucose concentration.

Results of continuous glucose monitoring up to 72 hours in healthy and diabetic volunteers have been described (Pfeiffer *et al.*, 1993). Following an oral intake of carbohydrate, a time-lag of about 20 minutes was observed. Taking this together with the dead volume of the tubing into account, good correlations with the i.v. and the subcutaneous glucose were obtained. Another study by the same group was designed to estimate the real glucose concentration in the subcutaneous tissue of healthy volunteers (Sternberg *et al.*, 1995). It was based on recirculation of the perfusing solution in the microdialysis probe until complete equilibration was achieved between the glucose outside and inside the probe. Using a recirculation rate of 2.5 $\mu\text{L}/\text{min}$ and a number of cycles ranging between 33 and 52 (cycle duration = 3.7 min), the subcutaneous glucose concentration measured was 72 ± 6 and $78 \pm 6\%$ of the blood glucose measured in the fasting state and during a hyperglycaemic clamp, respectively.

Short-term experiments on non-diabetic subjects following an OGTT showed good correlation of the blood and dialysate glucose values (Moscone *et al.*, 1992). A sharp decrease in sensitivity was observed after two hours of implantation: this was explained by the release, during the inflammatory reaction, of an interfering compound which reacted with the enzyme or electrode. A complete system has been investigated—the perfusion solution reservoir, a waste bag and micropump were integrated into one unit and the electronics into a second one (Hashiguchi *et al.*, 1994). After evaluation of the system in animals for seven days, it was tested on five healthy and eight diabetic patients. Perfusion rates of 2 $\mu\text{L}/\text{min}$ were used and the system was calibrated *in vivo* prior to the experiment. The calibration factor could be used for up to four days of implantation during which close agreement between blood glucose values and glucose concentrations in the dialysate was obtained. Due to the decrease of the recovery after four days of implantation, the recalibration either had to be performed daily (i.e. on days 5, 6, 7, 8) or the hollow-fibre probe had to be changed. The decrease of the recovery was also

observed in saline solutions containing 10–50 g/L albumin. The biofouling of the hollow-fibre by various proteins was visible under the scanning electron microscope. By recalibrating the system, glucose monitoring could be performed for up to seven days.

The approach adopted by Aalders *et al.* (1991) was different, since it used a glucose oxidase-containing saline solution (0.15 mg/ml) for perfusion at 20 $\mu\text{L}/\text{min}$. The probe was subcutaneously implanted in non-diabetic and diabetic patients, and tested intermittently up to nine days. Good correlation between the dialysate and the blood glucose values was obtained, with time-lags of 4.4 and 8.2 minutes for rising and decreasing glucose concentrations for the non-diabetics, and a maximum delay of 22 minutes for the diabetic patients. In order to decrease the risk of the enzyme leakage into the body, a more sophisticated, double-membrane, hollow-fibre microdialysis probe was developed (Schmidt *et al.*, 1992). It was tested in 12 non-diabetic and 12 diabetic patients using steady-state glucose clamps, allowing two-point calibration of the sensor to be performed. The *in vivo/in vitro* coefficients for both non-diabetic and diabetic patients correlated well, with low inter-individual variations. The conclusion of this work was that when *in vitro* calibration factors are used, the sensor estimated subcutaneous tissue glucose to be $43 \pm 9\%$ of the blood glucose value.

2.7 REVERSE IONTOPHORESIS

Iontophoresis is a well established technique, which uses an externally applied potential difference (electric field) to promote molecular transport across the skin. Interest in this technique is increasingly motivated by the possibility of performing controlled drug delivery via the transdermal route. The mechanisms and the kinetics of fluxes across the skin (electromigration, electro-osmotic and osmotic fluxes) are quite complex (Burnette, 1989). In brief, while an enhanced flux of charged molecules is due to electromigration, the concomitantly observed enhancement of the flux of neutral molecules is attributed largely to electro-osmotic flow. The skin is believed to be negatively charged at $\text{pH} > 4$ and is therefore permselective to cations: this provokes a pre-dominant electro-osmotic and osmotic flow of solvent from anode to cathode. The flow can be used to enhance transdermal flux of glucose in the subcutaneous extracellular fluid, thus allowing minimally-invasive glucose monitoring. The transdermally-extracted samples seem to be less aggressive to the sensor than blood or subcutaneous tissue itself, and thus less problems from biofouling might be expected. However there are several points that need to be clarified before considering long-term use of transdermal glucose monitoring by reverse iontophoresis. Among them are the long-term effects of the current on the skin permeability and biology; site-to-site variability of glucose recovery; response time (at least 15 minutes are needed to extract a

volume containing a measurable amount of glucose) and, as is the case for most implantable devices, the frequency of sensor recalibration.

The feasibility of reverse iontophoresis for glucose monitoring has been demonstrated *in vitro* using mouse skin (Rao *et al.*, 1993). Iontophoresis was performed for two hours at a current density of 0.36 mA/cm^2 , and the amount of glucose was measured using either a classical enzyme electrode or a modified copper electrode. Using the former, a good correlation was obtained between subdermal (1–18 mM glucose range) and extracted (cathodic compartment) glucose concentration. Using two 0.4 ml extraction chambers attached to the skin of diabetic patients, the feasibility of the transdermal glucose monitoring has been demonstrated (Tamada *et al.*, 1995). Extracted glucose concentrations closely followed the changes in blood glucose levels induced by an OGTT, followed by the administration of insulin (albeit with a time-lag of 20 minutes). In this case, an HPLC was used for glucose assay. A current of 0.32 mA/cm^2 was applied during 15 minutes, with a reversal of the polarity at half-cycle. The extracted glucose values were correlated with blood glucose levels measured every 7.5 minutes. Using Clarke error grid analysis, good correlation between blood and transdermally extracted glucose concentrations was obtained for all five subjects and 12 experiments. Minor skin irritations were observed at the test site immediately after removal of the extraction chambers.

Experiments performed on non-diabetic subjects, using 0.25 mA/cm^2 and extraction chambers of 0.55 ml, have confirmed the feasibility of reverse iontophoretic glucose monitoring (Rao *et al.*, 1996). Chromatography with pulsed amperometric detection was used for glucose analysis. Investigating the influence of the extraction time on the correlation of the subdermal and extracted glucose concentrations showed inconsistent results for short extraction times (approx. 15 min). Measurements of the passive glucose diffusion (i.e. without applied current) showed high initial extractions of glucose. It was postulated that this glucose comes from the stratum corneum where it was produced by deglycosylation of glucosylceramides and that it is not correlated with the systemic concentration. With sampling times of 1 h, good correlations were obtained, although in absolute terms these differed between individuals. For longer extraction times, slight skin irritation was also observed.

2.8 CONCLUDING REMARKS

The development of short- and long-term glucose sensors remains a key target of *in vivo* monitoring. Miniaturisation of the amperometric enzyme electrodes already used successfully in laboratory analysers and in disposable self-test strips has appeared as the most straightforward technological choice.

Although not yet entirely solved, the technological aspect of producing reliable sensors (see Chapter 7) does not constitute the most critical issue hindering the introduction of sensors into clinical practice. Different sensor configurations have been shown to possess the required *in vitro* characteristics, including reasonable functional and storage lifetimes. In the case of implantable sensors, the main challenge concerns the improvement of the sensor's biostability. No consensus has been reached on how to achieve this goal, for either tissue or intravenous implantation. This issue concerns the microdialysis probes which are also subject to biofouling, leading to a change of recovery with implantation time. Although the feasibility of reverse iontophoresis has been demonstrated, detailed investigation of its long-term effect on skin permeability must be carried out before deciding on the clinical usefulness of the method. As can be seen for all the approaches described above, there are fundamental questions—by no means trivial—that require urgent answers.

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